Research Article
Future Microbiology

Antimicrobial activity, cytotoxicity and inflammatory response of novel plastics embedded with silver nanoparticles

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Aim: Infections associated with medical devices are an important cause of morbidity and mortality. Microorganisms are responsible for catheter infections that may then result in the local or systemic dissemination of the microorganism into the bloodstream. The aim of this study was to evaluate the antimicrobial activity of silver nanoparticles (AgNPs) embedded in polyurethane plastics, commonly used for catheter fabrication. **Materials & methods:** AgNPs in the range of 25–30 nm were synthesized and embedded in polyurethane plastics at different concentrations. The antimicrobial activities of these plastics were tested against the three pathogenic microorganisms, *Escherichia coli, Staphylococcus epidermidis* and *Candida albicans,* frequently associated with catheter infections. The cytotoxicity of the plastics was evaluated on human-derived macrophages using propidium iodide and the secretion of the pro- and anti-inflammatory cytokines IL-6, IL-10 and TNF-α was measured using ELISA. **Results:** A significant reduction of 6- to 7-log in the number of bacteria was measured, while a reduction of 90% was measured in the case of *C. albicans*. Neither cytotoxic effect on macrophages nor immunological response was observed. **Conclusion:** Plastics embedded with AgNPs have great potential to limit microbial colonization of implanted medical devices.

Colonization of medical devices by microorganisms followed by the establishment of infection represents an increasing burden to healthcare owing to the appearance of antibiotic-resistant pathogenic and opportunistic microorganisms. Of special interest are nosocomial infections from medical devices, which continue to pose a significant problem for patients in intensive care units, despite aggressive education for healthcare personnel [1,2].

Infections associated with central venous catheters, commonly used for intravenous administration of fluids and medicines, are an important cause of morbidity and mortality in ambulatory and hospitalized patients [3]. Central venous catheters assure vascular access and their use is extended to intensive care unit patients, long-term indwelling catheter for cancer patients, total parenteral nutrition and hemodialysis, among others. The major risk associated with these devices is microbial infection, as they constitute a direct pathway between the external environment and the bloodstream [4].

Microorganisms are responsible for catheter infections if they are able to colonize and establish a biofilm on the medical device after gaining access to the surface of the implanted catheter. The subsequent local or systemic dissemination of these microorganisms into the bloodstream possesses a serious health risk to the patient. Although the most effective way to prevent catheter-related bloodstream infections is the insertion of catheters under sterile conditions [5,6], different routes for microbial contamination can occur over short- and long-term use of these intravascular devices [7,8].

Silver nanoparticles (AgNPs) have emerged as an alternative to conventional antimicrobial agents owing to their remarkable antimicrobial activity [9–12], and they are attractive compounds for use in medical applications. For instance, AgNPs are included in the production of

Keywords
■ antimicrobial activity ■ cytotoxicity ■ immunological response ■ medical devices ■ silver nanoparticles

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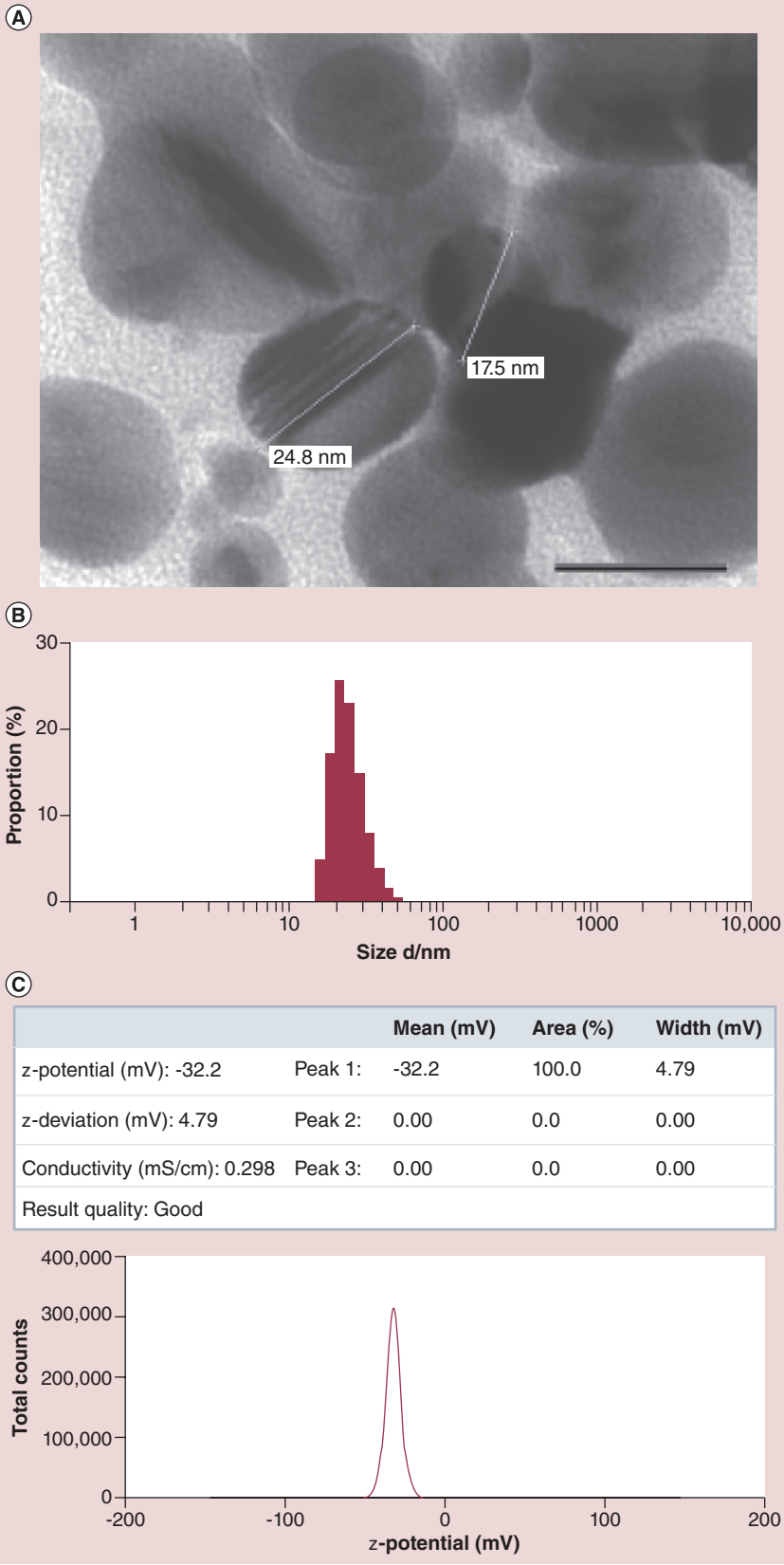

		Mean (mV)	Area (%)	Width (mV)
z-potential (mV): -32.2	Peak 1:	-32.2	100.0	4.79
z-deviation (mV): 4.79	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.298	Peak 3:	0.00	0.0	0.00
Result quality: Good				

Figure 1. Physical characterization of synthesized silver nanoparticles, with an average size of 25–30 nm. (A) Transmission electron micrograph of the nanocomposite. Scale bar represents 20 nm. **(B)** Distribution of the nanoparticles according to size using dynamic light scattering. **(C)** ζ-potential distribution.

wound dressings, contraceptive devices, surgical instruments and bone prostheses [13]; thus, the inclusion of AgNPs in catheters can provide additional protection against colonization and infections in the short- and long-term use of these medical devices [14].

The mechanisms of antimicrobial toxicity have not yet been elucidated; however, it seems to be the interaction of several factors. For example, Ag ions can be released from the oxidation of AgNPs and deposited on the microbial cell wall [15]. As a result of the interaction of these Ag ions, proteins can be inactivated [16]. However, other studies reported the penetration of AgNPs into the cell wall [12], which can cause a change in cell wall permeability, affecting the normal transport of metabolites and electrolytes [17]. Moreover, the high affinity of Ag ions for sulfur and phosphorous may generate complexes detrimental for the proper function of components on the cell wall [18,19].

The mechanism involved in the toxicity caused to mammalian cells includes the enhancement of reactive oxygen species generation, which can alter the catalysis of biological reduction–oxidation processes. As a result, exposure of cells to AgNP may produce cellular signaling cascades that control cellular proliferation, inflammatory processes and cell death [20]. Thus, AgNPs exposed to biological systems can be corroded, degraded or dissolved into Ag ions interacting with proteins [21]. Also, the exposure of AgNPs to mammalian cells can produce a significant increase in DNA fragmentation [22].

In this study, the antimicrobial activity of plastics embedded with AgNPs on three pathogens commonly associated with catheter infections were evaluated. In addition, the cytotoxicity and inflammatory response of these plastics were assessed on a human-derived monocyte cell line by measuring the integrity of macrophage membranes and the secretion of the cytokines IL-6, IL-10 and TNF-α.

Materials & methods

Synthesis & characterization of AgNPs

AgNPs were synthesized and characterized as published [9]. All the characterization analyses were performed in aqueous suspensions.

Production & characterization of plastics embedded with AgNPs

AgNPs in the range of 25–30 nm were precipitated with HNO_3 (Sigma-Aldrich, MO, USA). The precipitate was filtered, washed with double-distilled water and dried at room temperature.

Two groups of plastics were prepared by dissolving the resin 4,4′-diphenylmethane diisocyanate (BASF Corporation, NJ, USA) in either EtOH 96% (Sigma-Aldrich) or acetone (Sigma-Aldrich). Plastics with AgNP concentrations varying from 0.05–0.7% were embedded (Table 1), then dried at room temperature for 2 days and cut into 3 cm^2 pieces. Plastics without the addition of AgNP were prepared as a negative control.

Synthesized plastics containing AgNPs were characterized by high-resolution scanning electron microscopy on a Hitachi S4800 microscope (ON, Canada).

Table 1. Composition of plastics embedded with silver nanoparticles.

Silver (%)	Silver weight (g)	Water (µl)	Dissolvent (µl)		Resin (g)	Ethylene glycol (µl)
			EtOH	*Acetone*		
0.05	0.012	200	750	–	24	1200
0.08	0.0192	300	1250	–	24	1200
0.1	0.024	400	1500	–	24	1200
0.3	0.072	750	1500	–	24	1200
0.5	0.12	1000	–	1500	24	1200
0.7	0.168	1000	–	1500	24	1200
Control	–	–	–	–	12	600

Decontamination of synthesized plastics

The decontamination process was performed in a laminar biological safety hood to maintain sterile conditions. Plastics were cut into 1 cm^2 pieces and placed in sterile petri dishes (6 cm diameter), soaked in 6 ml of 70% ethanol and gently agitated on a rocker at 75 rpm for 2 h. Plastic pieces were then transferred to empty sterile petri dishes and left open for 30 min to allow volatilization of the ethanol.

Microbial strains & culture media

The antimicrobial activities of the synthesized plastics were evaluated on *Escherichia coli* (ATCC 25922), *Staphylococcus epidermidis*

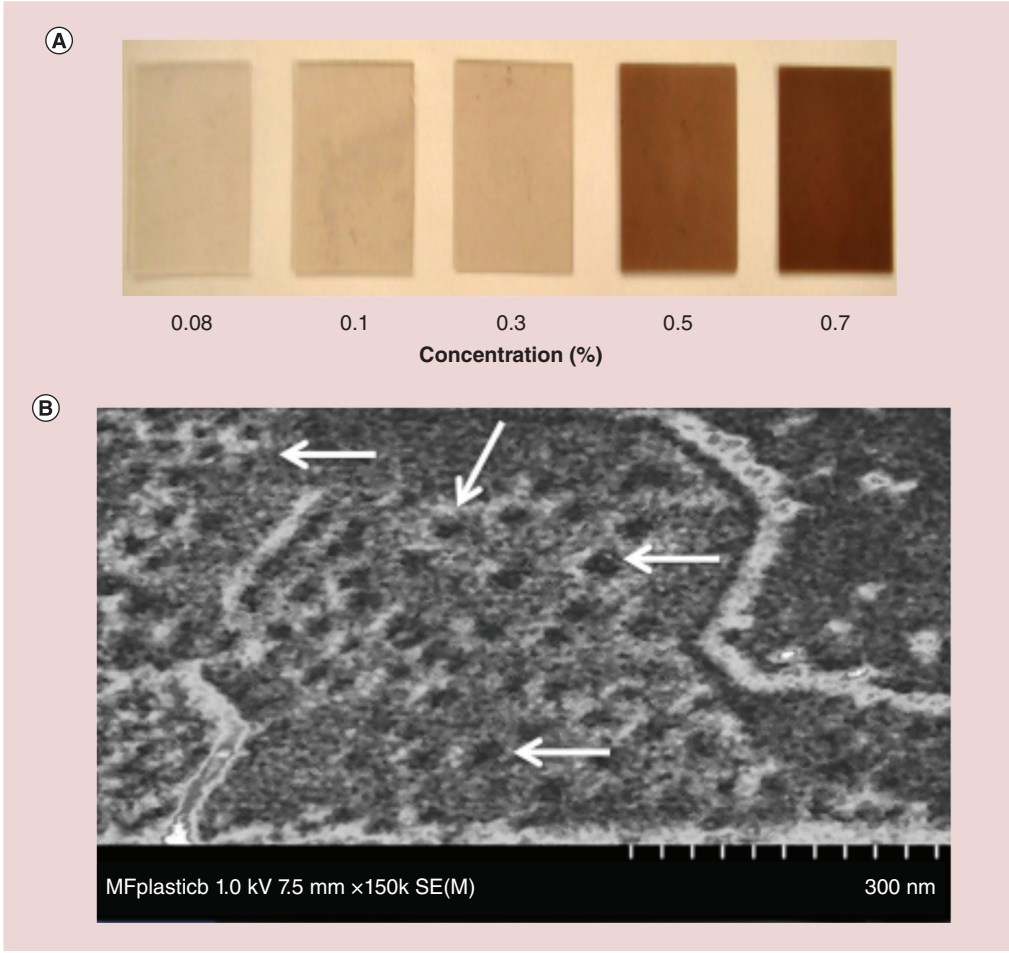

Figure 2. Synthesized plastics containing silver nanoparticles at different concentrations. **(A)** Examples of the plastics embedded with silver nanoparticles (NPs). **(B)** Scanning electron microscope image showing the distribution of silver NPs in the plastic. White arrows point to NPs.

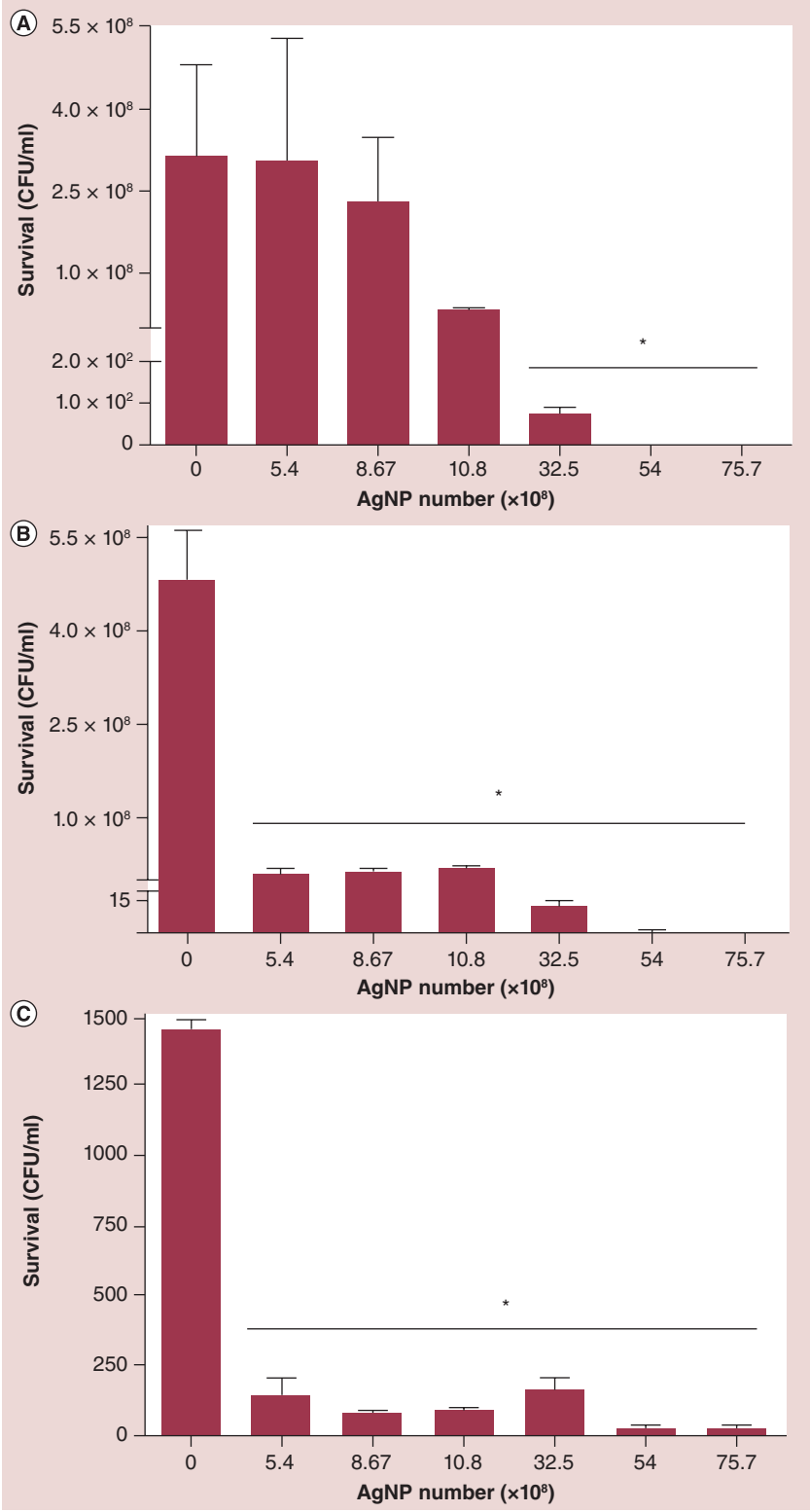

Figure 3. Antimicrobial activity of plastics embedded with different concentrations of silver nanoparticles. The pathogens commonly associated with catheter infections – **(A)** *Escherichia coli*, **(B)** *Staphylococcus epidermidis* and **(C)** *Candida albicans* – were exposed to plastics and their survival was evaluated after 24 h by CFU counting. The number of AgNPs is expressed per cm^2 of plastic. The mean ± standard deviation are shown for three independent experiments. *$p < 0.05$.
AgNP: Silver nanoparticle.

(ATCC 14990) and *Candida albicans* (ATCC 14053). *E. coli* and *S. epidemidis* strains were grown in brain heart infusion broth (BD Biosciences, CA, USA), and *C. albicans* was grown in Yeast Nitrogen Base (BD Biosciences). Fresh cultures were diluted with their respective broth to a concentration of 30 CFU/ml. This concentration was chosen since >15 CFU/ml is the accepted threshold used to model infection of central venous catheters [23].

Antimicrobial activity of synthesized plastics

Sterile plastics, either embedded with AgNPs or not, were transferred to sterile plates (6 cm diameter) and gently agitated with 6 ml of the respective broth, containing 30 CFU/ml in a rocker at 75 rpm for 24 h at 37°C. At timepoints of 0 and 24 h, aliquots were diluted and plated on brain heart infusion agar or Yeast Nitrogen Base agar. The plates were incubated at 37°C for 24 h and the CFU/ml was determined. Each plastic was assayed in triplicate.

Cytotoxicity of synthesized plastics on macrophages

The human-derived monocytic cell line THP-1 (ATCC TIB202) was used to assess cytotoxicity and the inflammatory response. Macrophages were grown in RPMI 1640 (Hyclone, UT, USA) supplemented with 5% fetal calf serum (Hyclone) and 2 mM L-glutamine (StemCell Technologies, BC, Canada). Cell viability was assessed by Trypan blue (Sigma-Aldrich) exclusion and cells were only used when viability exceeded 95%. Macrophages became adherent to the bottom of the wells after incubation with 20 ng/ml phorbol 12-myristate 13-acetate (Sigma-Aldrich) [24]. A 24-well plate format was used and incubated overnight at 37°C in an atmosphere supplemented with 5% CO_2. The following day, plastic pieces (1 cm^2) were placed in 24-well plates in sterile conditions. Differentiated THP-1 cells at a density of 1×10^6/per well, were dispensed. Plates were incubated at 37°C in a humidified atmosphere supplemented with 5% CO_2 for 24 h. THP-1 cells treated for 6 h with 5% H_2O_2 were used as positive controls, while untreated cells were used as negative controls. After 24 h incubation, plastics were transferred aseptically to a new 24-cell plate and washed with 400 µl of RPMI 1640 with gentle rocking at 100 rpm for 30 min. Finally, plastics were transferred to another 24-cell plate, where they were trypsinized with trypsin (0.025%) for 10 min.

These suspensions were transferred to 1.5 ml conical microcentrifuge tubes, and washed with 500 µl of RPMI 1640 by centrifugation at 500 rpm for 5 min. The supernatants were removed and washing was repeated twice more. The toxicity of the plastics was measured by staining macrophages with propidium iodide (Sigma-Aldrich) and according to published protocols [25]. Propidium iodide emission was detected at 610–625 nm using the FL3 gate of a BD FACS Vantage SE Turbo sort cell sorter (BD Biosciences). Experiments were performed three times.

Inflammatory response

Based on the cytokine expression level of differentiated THP-1 cells, the inflammatory response was evaluated by ELISA. After 24 h of exposure of macrophages to the plastics containing 0.3, 0.5 and 0.7% AgNPs, the concentrations of IL-6 (BD Biosciences), IL-10 (BD Biosciences) and TNF-α (eBiosciences, CA, USA) were measured in supernatants. The level of endotoxins was assessed by Limulus Amebocyte Lysate kit (MA, USA) according to the instructions of the manufacturer. Experiments were performed three times.

Presence of colonization on the surfaces of plastics with AgNPs

Plastic pieces of 1 cm^2 were immersed in 24-well plates containing 100 CFU/ml of each assessed strain, diluted in 1 ml of broth. After 24 h incubation at 37°C, plastics were washed twice with sterile double-distilled water and aseptically transferred to 10 ml of PBS. Each plastic was vortexed for 30 s, sonicated for 1 min and vortexed for a further 30 s. Serial dilutions were prepared from the supernatants, then plated and the CFU number was determined the next day after incubation at 37°C. Plastics were also exposed to the microbial strains for 72 and 96 h, and the medium was replaced with fresh medium every 24 h. Experiments were performed three times.

Results

Synthesis & characterization of AgNPs & plastics

AgNPs were synthesized and their physical characteristics were analyzed. A particle size distribution between 25–30 nm was measured with a ζ-potential of -32.2 ± 4.79 mV (Figures 1A–C). These AgNPs were embedded into plastics using AgNP concentrations between 0.05–0.7% (Table 1). The homogeneity and distribution of AgNP in the embedded plastics are illustrated in Figure 2.

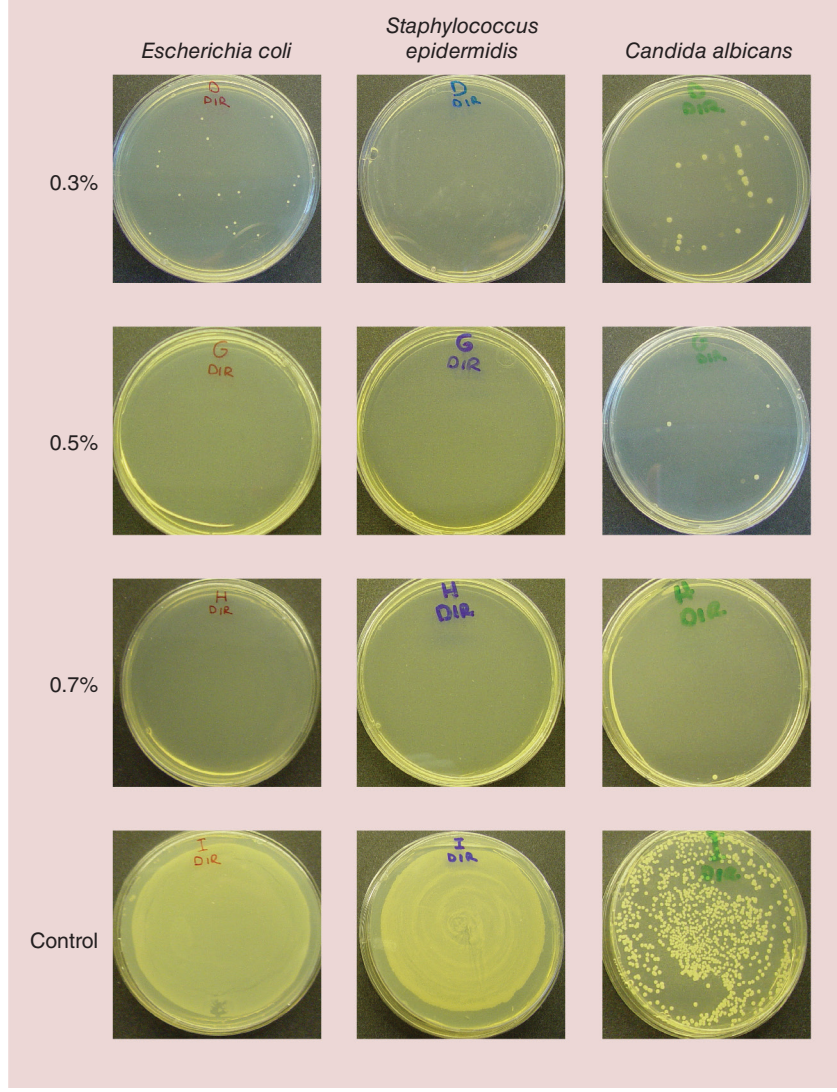

Figure 4. Survival of microorganisms exposed to plastics containing silver nanoparticles. Plates showing colony growth of pathogens after exposure to plastics are shown as an illustration. The control represents pathogens exposed to plastic containing no silver nanoparticles.

Antimicrobial activities

The antimicrobial activities of the synthesized plastics were evaluated on three common pathogens associated with catheter infections *E. coli*, *S. epidermidis* and *C. albicans*. A 6-log reduction in the CFU/ml number of *E. coli* was measured when the microorganism was exposed to plastics embedded with 0.3% AgNPs, while complete growth inhibition was observed with plastics containing >0.5% AgNPs (Figure 3A). In the case of *S. epidermidis*, a reduction of 7-log was observed when the pathogen was exposed to plastics with >0.05% AgNPs and, as in the previous case, complete growth inhibition was

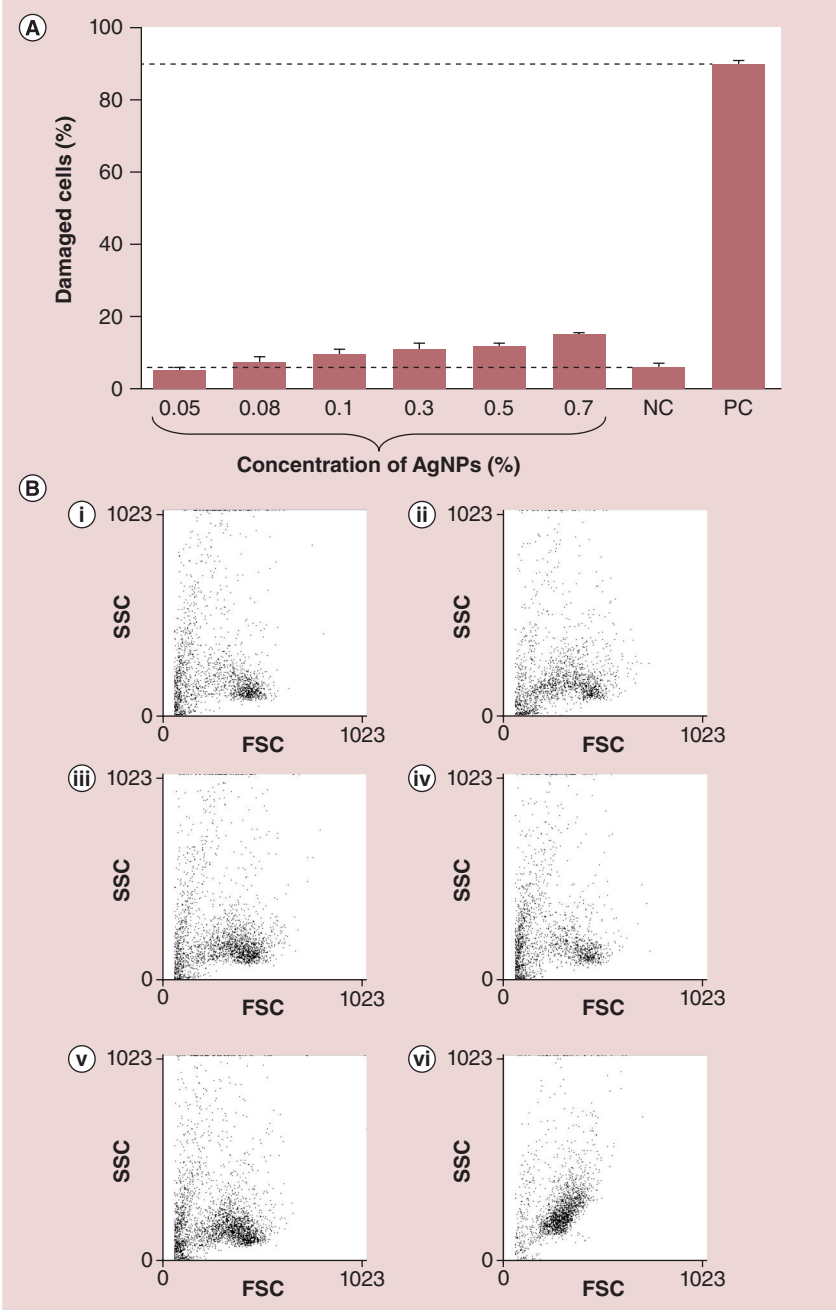

Figure 5. Cytotoxic effects of plastics on macrophages. (A) Determination of the cytotoxic effects of plastics on THP-1 macrophages after 24 h incubation. The dashed line represents treatment with 5% hydrogen peroxide, used as a PC. Untreated cells were used as a NC. The mean ± standard deviation are shown for three independent experiments. Plotted data refer to propidium iodide stain intensity detected on the FL3 channel. **(B)** Graphs showing the distribution of THP-1 exposed to plastics containing **(i)** 0.05%, **(ii)** 0.1%, **(iii)** 0.5% and **(iv)** 0.7% AgNPs. **(v)** Untreated THP-1 cells and **(vi)** THP-1 cells treated with 5% hydrogen peroxide were used as NCs and PCs, respectively. Plotted data refer to the linear FSC versus log SSC dot plots.
AgNP: Silver nanoparticle; FSC: Forward scatter; NC: Negative control; PC: Positive control; SSC: Side scatter.

observed when the pathogen was exposed to concentrations over 0.5% AgNPs (Figure 3B). Interestingly, although a reduction of over 90% in the CFU number was observed for *C. albicans*, upon exposure to >0.05% AgNPs, the highest concentration of AgNPs tested (0.7%) was not sufficient to avoid the growth of the fungus, as 2% of the starting number of the microorganism was able to grow after the exposure time (Figure 3C). An illustration of the log reduction of pathogens with AgNPs exposure is shown in Figure 4.

Cytotoxicity & LD_{50}

Differentiated THP-1 cells were used to determine the cytotoxic effects of plastics embedded with AgNPs. Plastics were assayed at 37°C for 24 h and cytotoxicity was measured using flow cytometry. No significant cytotoxic effects were measured even when macrophages were exposed to the highest concentration of AgNPs (15% macrophage death; Figure 5). In addition to this, a value corresponding to 0.805 g of AgNPs was calculated as the LD_{50}, suggesting that the use of these embedded plastics at the highest concentrations of AgNPs (0.7%, 0.168 g) is safe.

Inflammatory response

To determine whether the use of the synthesized plastics was able to stimulate an immunological response, the pro-inflammatory cytokines IL-6 and TNF-α, as well as anti-inflammatory IL-10, were analyzed in macrophages at the translation level using ELISA. No significant changes in the concentrations of the three cytokines were measured when compared with the control (Figures 6A–C), suggesting that the embedded plastics are not able to stimulate an immunological response.

Discussion

Nosocomial infections are one of the most serious complications in intensive care units because they lead to high morbidity, mortality, length of stay and overall cost of treatment. An estimated 80,000 bloodstream nosocomial infections associated with central venous catheters occur annually in the USA [6]; therefore, there is an urgent need for the development of novel technologies to reduce this burden. In previous studies, we reported that AgNPs possess antimicrobial activities against a panel of pathogens, including clinical strains [9,10]. Therefore, we investigated whether AgNPs embedded into plastics retained their antimicrobial properties.

The synthesis of AgNPs was performed as previously reported [9], and their size, distribution and shape were confirmed. After the inclusion of AgNPs in the fabrication of polyurethane-based

plastics, electron microscopy images revealed that the AgNPs were uniformly distributed (Figure 2). Similar homogeneous distribution of AgNPs was reported in polyester-type waterborne polyurethane [26] and previous studies reported an enhancement of the biostability, biocompatibility and bioactivity when AgNPs were embedded into plastics [27].

Several studies have reported the antimicrobial activity of AgNPs exposed to pathogenic microorganims, including clinical isolates [9–12]. Thus, AgNPs were embedded into polyurethane to evaluate their antimicrobial activity against microorganisms commonly associated with the colonization of catheters, such as *E. coli*, *S. epidermidis* and *C. albicans* [28]. Exposure of these microorganisms to the AgNPs plastics showed that AgNP concentrations of over 0.3% effectively inhibited the growth of the pathogens on the plastic surfaces almost completely(~6–7-log reduction), demonstrating their efficacy to control colonization and the growth of microorganisms. Other studies have previously reported a 1–3-log decrease in the number of *E. coli* bacteria exposed to different plastic compositions. These studies indicated that the highest antimicrobial activity (~3-log reduction) was observed in plastic poly(oxyethylene)-segmented imide (POEM), which contained AgNPs within the range of 10–30 nm in diameter [29], however in this study we report a higher log reduction using the same bacterial species. Taken together, these studies demonstrate that AgNPs can be used as efficient antimicrobial agents when embedded in different polymers.

Given the remarkable antimicrobial activity displayed by AgNPs embedded in plastic, we evaluated the potential cytotoxic effects caused by the plastics when exposed to macrophages at the concentration that showed antimicrobial activity. These cells were chosen as they constitute a first line of defense against pathogens and products of the tissue damage. Interestingly, plastics containing 0.05–0.7% AgNPs showed no cytotoxicity in human macrophages. Similar results had been previously reported when murine macrophages J774.2 were exposed to polystyrene- and polyvinyl-derived plastics embedded with AgNPs [29]; however, severe cytotoxicity was measured when POEM plastics containing AgNPs were exposed to the same murine macrophage cell line [29]. The authors of this study accounted for the differential cytotoxicity of POEM plastics by relating it to the abundance of amino groups or to the stabilizer used in POEM fabrication.

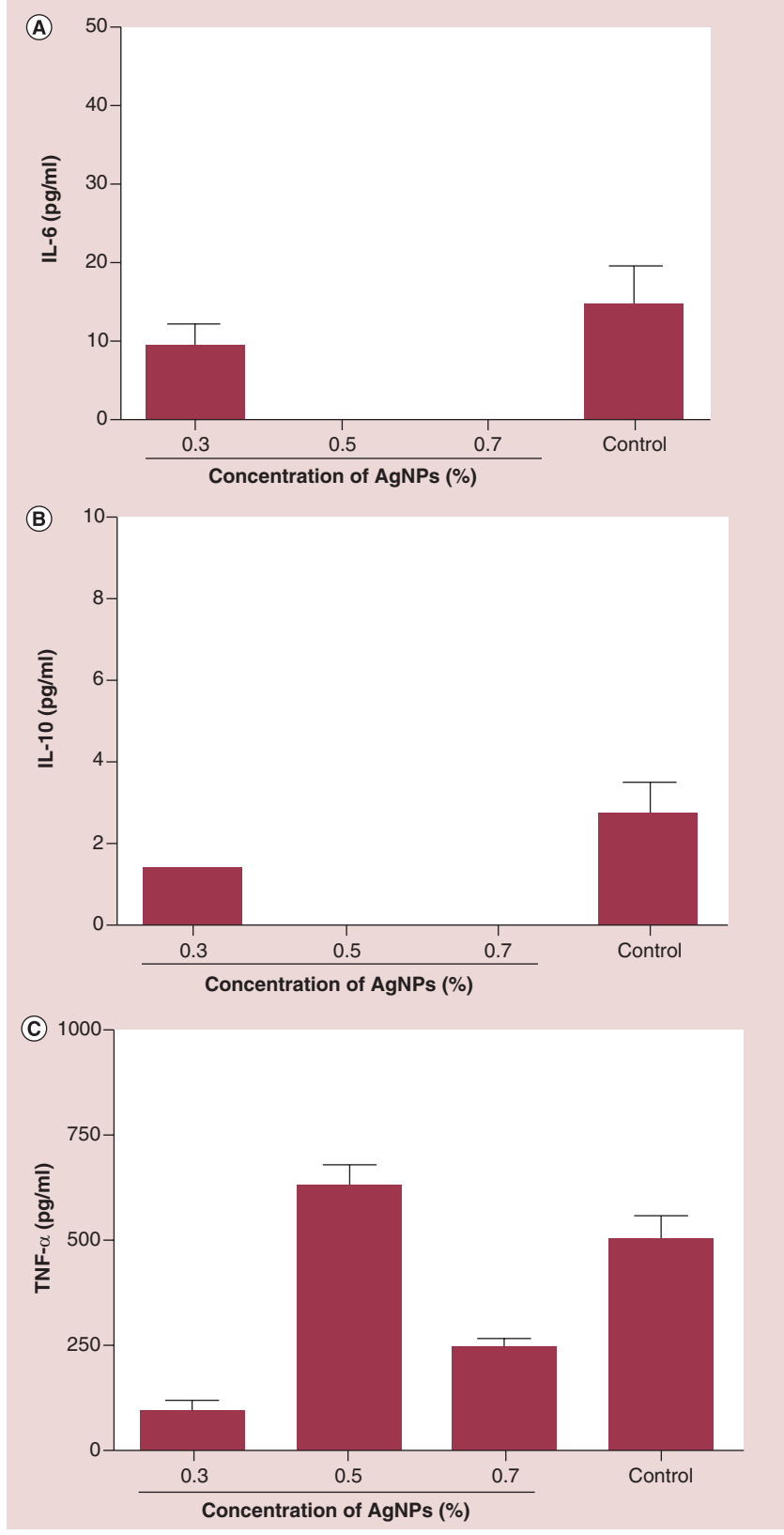

Figure 6. Analysis of inflammatory cytokines. THP-1 cells were incubated in the presence of plastics containing AgNPs and the levels of the secreted cytokines **(A)** IL-6, **(B)** IL-10 and **(C)** TNF-α were analyzed by ELISA after 24 h exposure. The mean ± standard deviation are shown for three independent experiments. AgNP: Silver nanoparticle.

Macrophages constitute the first defense line able to elicit an immunological response. To determine whether AgNPs embedded in polyurethane can elicit an immunological response, the secretion of pro- and anti-inflammatory cytokines at the protein level was evaluated using human macrophages. Surprisingly, our embedded plastics did not stimulate the secretion of IL-6, IL-10 or TNF-α. Previous reports have found that macrophages exposed to a suspension of AgNPs increased the release of IL-6 when the AgNPs were used at concentrations of 5 and 10 μg/ml [10], while the concentration of TNF-α reached significant values at a concentration of 10 μg/ml [10]. Interestingly, no changes in the secretion of IL-10 were observed when both AgNP concentrations were used. However, another study reported that exposure of AgNPs with diameters of 25, 40 and 80 nm induced significant secretion of TNF-α in primary rat brain microvessel endothelial cells [30]. Similar results for IL-6 and TNF-α were reported when 0.34 mg/ml of AgNPs with diameters of 20, 50 or 80 nm were exposed to human epidermal keratinocytes [31].

Although the preliminary cytotoxicity and inflammatory response data shown in this study suggest that these AgNPs can be used to avoid colonization of microorganisms, additional studies are required to show their biocompatibility regarding oxidative stress, inactivation of proteins and DNA fragmentation [20–22]. Recently, it has been reported that the release of Ag ions from AgNPs deposited on polytetrafluoroethylene polymer depends on the particle size. Interestingly, the authors were able to stabilize the morphology of the AgNPs and to control their Ag ion release rate by coating the AgNP surface with another layer of polytetrafluoroethylene devoid of AgNPs, which served as a polymer barrier [32]. Consistent with this report, the release of Ag ions from a biodegradable poly(DL-lactide-co-glycolide) copolymer was controlled by the polymer degradation process when immersed in PBS [33].

Taken together, the release of Ag ions from AgNPs constitutes a major health concern that should be addressed in studies involving AgNPs embedded in polymers.

Conclusion

We developed polyurethane plastics embedded with AgNPs. These plastics show compatibility to mammalian cells (human-derived macrophages) with no stimulatory effect on the inflammatory response. In addition, our study suggests that AgNPs, with a diameter ranging between 25–30 nm, have great potential as antimicrobial agents when embedded into plastics, thereby reducing the microorganism burden on medical devices.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Polyurethane plastics embedded with silver nanoparticles (AgNPs) were synthesized.
- The diameter of the AgNPs ranged between 25 and 30 nm.
- Plastics were assessed for their antimicrobial activity against three microbial strains associated with catheter infections.
- A significant antimicrobial activity against all the strains was measured.
- The inflammatory response of the plastics was evaluated by measuring the concentrations of IL-6, IL-10 and TNF-α in the supernatant of macrophages exposed to the plastics.
- No stimulatory effects on the inflammatory response were detected.
- Cytotoxicity was assessed by measuring the fluorescence of propidium iodide acquired by macrophages exposed to plastics.
- No cytotoxic effects were observed when these plastics were exposed to human macrophages.
- AgNPs have great potential as antimicrobial agents when embedded into plastics, thereby reducing the microorganism burden on medical devices.

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